Annals of Clinical and Analytical Medicine
Original Research

Epidermal growth factor receptor expression and adjuvant chemoradiotherapy in rectal cancer

Epidermal growth factor recepto in operated rectal cancer

Murtaza Parvizi[1], Derya Demir[2], Engin Kut[3], Erhan Ergin[4], Semin Ayhan[5], Başak Doğanavşargil[2]
[1] Department of Radiation Oncology, Manisa State Hospital, Manisa
[2] Department of Pathology, Faculty of Medicine, Ege University, Izmir
[3] Department of Medical Oncology, Manisa State Hospital, Manisa
[4] Department of Internal Medicine, Manisa State Hospital, Manisa
[5] Department of Pathology, Faculty of Medicine, Manisa Celal Bayar University, Manisa, Turkey

Abstract

Aim: Epidermal Growth Factor Receptor (EGFR) is a trans-membrane protein with tyrosine kinase activity and is expressed in 25-80% of colon cancer cases. EFGR expression is prognostic in patients with metastatic colorectal cancer, and anti-EGR- based therapies are routinely used in the treatment of patients with metastatic colorectal cancer. To the best of our knowledge, the relationship between EGFR expression and prognosis in directly operated patients who did not receive neoadjuvant treatment and subsequently received chemo-radiotherapy is unknown. Therefore, we retrospectively evaluated patients with stage 3 rectal cancer who underwent surgery without any preoperative treatment in our center and aimed to investigate the relationship between EGFR expression and prognosis in patients who received adjuvant chemoradiotherapy.

Material and Methods: The data of patients who underwent surgery for rectal cancer and received chemoradiotherapy between 2010 and 2016 at Manisa State Hospital were retrospectively analyzed.

Results: According to EGFR expression, it was 127.01 (95% CI, 85.43-168.59) months in the group with 10% less staining and 47.44 (95% CI, 26.77-68.12) months in the group with 10% or more staining. Lymphovascular invasion (p=0.032), perineural invasion (p=0.023), histologic grade (p=0.004) and EGFR expression percentage (p=0.005) were significantly associated with survival in multivariate analyses

Discussion: The presence of 10% or more EGFR expression, LVI, PNI, and histological grade are significantly associated with survival in stage 3 rectal cancer patients who have undergone surgery and received postoperative chemotherapy. These markers can be used as prognostic biomarkers in the follow-up and treatment of these patients.

Keywords

Epidermal Growth Factor Receptor, Chemoradiotherapy, Rectal Cancer, Prognosis

DOI: 10.4328/ACAM.21649 Received: 2023-02-10 Accepted: 2023-03-16 Published Online: 2023-03-19 Printed: 2023-03-25 Ann Clin Anal Med 2023;14(Suppl 1):S86-89
Corresponding Author: Murtaza Parvizi, Department of Radiation Oncology, Manisa State Hospital, 45040, Şehzadeler, Manisa, Turkey.
E-mail: 45onkoloji@gmail.com P: +90 532 604 06 40 F: +90 236 229 26 50
Corresponding Author ORCID ID: https://orcid.org/0000-0002-0280-7321
This study was approved by the Health Sciences Ethics Committee of Ege University (Date: 2017-01-27, No: 16-12.1/1)

Introduction

According to the Global Cancer Observatory (GLOBACAN), colorectal cancer is the 4th most common cancer in the world and ranks 3rd in terms of mortality. It is known that mortality rates are gradually decreasing in parallel with successful developments towards improving screening programs and treatment [1]. Similar to these developments, in the oncological statistics for 2017, a decrease in incidence and mortality rates was defined [2]. Epidermal Growth Factor Receptor (EGFR) is a trans-membrane protein with tyrosine kinase activity and is expressed in 25-80% of colon cancer cases [3]. In addition to being known to be more highly expressed in left colon tumors, EGFR expression has been reported to be associated with cancer cell proliferation, apoptosis, angiogenesis, tumor invasion and distant metastasis [4], and therapies targeting EGFR in metastatic patients are currently used in daily practice in lung, colon and head and neck cancers. Currently, neoadjuvant radiochemotherapy is recommended as the standard treatment for patients with clinical >T3 or lymph node-positive rectal cancer [5]. However, in addition to the prejudice against radiotherapy, fear and anxiety about the progression of the disease during the neoadjuvant treatment period, surgery may sometimes be preferred as the first treatment method in rectal cancer cases due to the preference of the patients, the presence of clinical symptoms or the presence of conditions such as ileus, bleeding, etc., that require urgent surgery, or the difference between the radiological stage and the actual pathological stage.

To the best of our knowledge, the relationship between EGFR expression and prognosis in directly operated patients who did not receive neoadjuvant treatment and subsequently received chemo-radiotherapy is unknown. Therefore, we retrospectively evaluated patients with stage 3 rectal cancer who underwent surgery without any preoperative treatment in our center and aimed to investigate the relationship between EGFR expression and prognosis in patients who received adjuvant radiotherapy/radiochemotherapy.

Material and Methods

Study Population

Between 2010 and 2016, 89 patients diagnosed with "Adenocarcinoma of the Rectum" at Manisa State Hospital were retrospectively reevaluated. Among these patients, 50 patients who received neoadjuvant treatment were excluded and 39 patients with stage 3 who received postoperative adjuvant treatment were included in the study.

Data collection

Morphological and clinical prognostic parameters (age, gender, tumor location, tumor size, histological grade of tumor, postoperative TNM stage, perineural invasion (PNI), lymphovascular invasion (LVI), EGFR staining status and severity) were evaluated. The relationship between defined prognostic findings and survival (disease-free survival, overall survival, recurrence, metastasis development, serum CEA, CA19-9 levels at the time of diagnosis) values, EGFR expressions were retrospectively analyzed.

EGFR expression was investigated by immunohistochemistry in rectal adenocarcinoma patients who received adjuvant treatment and evaluated in comparison with morphologic, clinical prognostic parameters and survival time. Overall survival was defined as the time from the date of diagnosis until death. Disease-free survival was defined as postoperative disease progression or death due to any cause.

The hematoxylin-eosin stained slides of the cases were removed from the archive and re-evaluated and blank sections were prepared for immunohistochemical examination from the blocks of the selected appropriate slides (sufficient tumor in the biopsy and/or surgical material). Immunohistochemistry was performed automatically on 3-5 micron thick sections prepared from formalin-fixed paraffin blocks of the cases using EGFR antibody on a Leica BondMax immunohistochemistry device. EGFR (clone: EGFR.113, Leica-Novocastra, 1:20 dilution) primary antibodies were investigated by immunohistochemistry. The severity and intensity of positive staining were evaluated. Membranous and/or cytoplasmic staining was considered positive. EGFR staining intensity and intensity were interpreted semi-quantitatively (staining intensity +1, +2 and +3 positive; staining intensity "negative", "less than 10% positive", "10%-49% positive", "50% or more positive"). EGFR staining and evaluation were performed on the resection material and evaluated.

Ethical approval

The study was conducted in accordance with the principles of the Declaration of Helsinki and reviewed and approved by the Health Sciences Ethics Committee of Ege University (Approved with reference number 70198063-0500.06.04 and identification number 16-12.1/1, Date: 27/01/2017)

Statistical analysis

Descriptive statistics were presented as mean, standard deviation, median, minimum and maximum values for numerical variables and as numbers and percentages for categorical variables. Survival analyses were performed using the Kaplan-Meier method. Factors affecting survival were examined using the Cox regression. $p < 0.05$ was considered significant in all statistical analyses.

Ethical Approval

Ethics Committee approval for the study was obtained.

Results

Thirty-nine patients were included in this study. The pathology blocks of 11 patients were excluded from the study because of deterioration due to poor storage conditions and the remaining 28 patients were analyzed. Of the patients, 13 (46.4%) were female and 15 (53.6%) were male. The mean age of the patients was 60.93 (± 9.86) and is summarized in Table 1. All patients in the study received oral capecitabine chemotherapy concurrently with adjuvant radiotherapy followed by adjuvant 6 months of capecitabine oxaliplatin. At the time of evaluation, the median follow-up period was 60 (18.06-168.59) months, during which 2 (7.14%) patients developed local recurrence, 5 (17.86%) patients developed liver metastasis, 4 (14.29%) patients developed lung metastasis, 2 (7.14%) patients developed lymph node metastasis, and 1 (3.57%) patient developed brain metastasis. The presence of EGFR Expression was under 10% in 11(29.95%) patients and 10% or more in 17 (71.05%) patients.

Overall survival was defined as 59.63 (95% CI, 18.08-108.38)

months in the group with 10% or more staining. According to EGFR expression, it was 127.01 (95% CI, 85.43-168.59) months in the group with 10% less staining and 47.44 (95% CI, 26.77-68.12) months in the group with 10% or more staining. In univariate analyses, survival was significantly associated with histologic grade (p=0.006), N1 stage (p= 0.036), N2 stage (p=0.002), LVI (p=0.012), PNI (p=0.011) and EGFR expression percentage (p=0. 009) were identified as significant, while LVI (p=0.032), PNI (p=0.023), histologic grade (p=0.004) and EGFR expression percentage (p=0.005) were significantly associated with survival in multivariate analyses (Table 2) (Figure 1).

Table 1. Demographic, clinical and pathologic features of all patients.

		Number	%
Sex	M	15	46.4
	F	13	53.6
Histology	Adenocarcinoma	26	92.9
	Mucinous	1	3,6
	Ring signet cell	1	3.6
Grade	1 and 2	20	71.43
	3	8	28..57
Localisation	1-7 cm	9	32.1
	≥7	19	67.9
pT	pT4	5	17.9
	pT3	23	82.1
pN	N0-1	22	78.6
	N2	6	21.5
LVI	(+)	20	71.4
PNI	(+)	20	71.4

LVI: lymphovascular invasion; PNI: perineural invasion

Table 2. Univariate and multivariate analyses of overall survival

	Univariate (HR, 95% CI)	P value	Multivariate (HR, 95% CI)	P value
Age	1.03 (0.99-1.07)	0.154		
Sex	-0.41 (0.16-1.09)	0.77		
Grade	3.20 (1.78-4.82)	0.006	2.92 (2.007-5.23)	0.04
N2	3.38 (1.32-4.67)	0.002	3.50 (2.23-5.35)	0.02
N1	1.22 (1.18-5.35)	0.036	1.40 (0.97-4.31)	0.056
T3	2.88 (0.47-10.17)	0.32		
T4	2.16 (0.447-3.32)	0.66		
LVI	2.65 (1.47-4.37)	0.012	2.75 (1.10-4.64	0.032
PNI	2.59 (1.40-4.42)	0.011	2.65 (1.63-4.86)	0.023
Localisation	1.40 (0.58-3.41)	0.455		
Cea	-0.98 (0.94-1.04)	0.560		
CA19-9	1.01 (0.98-1.06)	0.252		
EGFR	2.82 (1.45-4.32)	0.009	2.86 (1.81-4.41)	0.005

Hazard ratio, HR; LVI, lymphovascular invasion; PNI, perineural invasion; CEA, Carcinembryonic antigen; CA19-9, carbohydrate antigen 19-9; EGFR, epidermal growth factor receptor

Discussion

When we look at the studies conducted on patients receiving radiotherapy in rectal cancer, since preoperative radiochemotherapy or radiotherapy is the standard treatment in locally advanced diseases, most of the studies include patients receiving preoperative treatment, but sometimes in daily practice, patients can be operated directly for some reasons and then receive adjuvant radiotherapy. We conducted this study to examine the relationship between EGFR expression and prognosis in this group. In our study, we found a relationship between eEGFR expression and the prognosis of patients with stage 3 rectal cancer who received adjuvant chemoradiotherapy.

Studies started with the first identification of epidermal growth factor (EGF) as an eye-opening protein in baby mice by Staley Cohen in 1962, followed by the identification of the EGFR receptor in 1975 and the determination of increased phosphorylation and tyrosine kinase activity when squamous cell carcinoma cells bind to EGF in the 1980s, began to take its place in clinical oncology [6]. EGFR is a transmembrane protein with tyrosine kinase activity and has been investigated in many cancers and its overexpression has been reported to be associated with proliferation, apoptosis, angiogenesis, tumor invasion and distant metastasis in many tumors [4]. Anti-EGFR-based therapies targeting EGFR in metastatic disease are widely used in colon, lung and head and neck tumors. Studies have also reported that patients with EGFR overexpression have more advanced-stage disease, worse histologic grade and lymphovascular invasion [7]. Longer survival times are observed with treatments including anti-EGFR therapy compared to those without [8-9]. While EGRF expression was defined between 25-80% in colon cancers in different studies, this rate was reported to be around 50-60% in rectal cancers [10]. Despite the observed EGRF expression, EGRF expression was found to be associated with prognosis in some studies involving patients with colon cancer independent of tumor stage, while no association with prognosis was defined in others [10-12]. It has been reported that the reason for the different results in EGFR-prognosis studies and the 25-80% difference between studies on EGFR-EGFR expression is related with the difficulties in the evaluation of staining, kit difference, center difference and staining difficulties in pathology samples in retrospective studies in which old blocks were evaluated [10]. In our study, staining was not detected in 28% of the patients due to storage conditions of old preparations, tissue loss during sectioning, etc. Huang et al. studied non-metastatic patients who were only operated and received adjuvant chemotherapy, and found a correlation between EGRF expression and prognosis in stage 3 colorectal cancers. They reported the presence of EGFR expression as a negative predictive factor for disease recurrence [11].

Previous studies have reported that EGR expression activates intracellular communication pathways, causing malignant transformation and tumor progression through increased cell proliferation, long-term survival, angiogenesis, antiapoptosis, invasion and metastasis. In studies on radiotherapy, it was reported that after radiotherapy, radiotherapy was associated with the presence of resistant tumor, and patients with EGFR overexpression had more local recurrence, lower stage and worse prognosis [3, 7-15].

Although the standard treatment for stage III rectal cancer is neoadjuvant radiochemotherapy/radiotherapy, stage III patients are rarely referred for postoperative adjuvant treatment in

some cases. In our study, we found that patients with EGFR expression above 10% had shorter survival times, and this group of patients had a poor prognosis similar to patients who received preoperative radiochemotherapy/radiotherapy.

We also confirmed the presence of a poor relationship between LVI and PNI and prognosis in our study in accordance with the literature (16-21), but we concluded that the lack of a relationship with T, N stage and histologic grade, which we know are normally associated with prognosis in colorectal cancers, may be due to the small number of cases and the small number of patients between the groups (for example, the number of patients with grade 1 was only 3).

The retrospective nature of our study and the small number of patients constitute the weaknesses of our study. However, in addition to the small number of patients, we believe that it will be beneficial to the deficiency in the literature on this subject. Our study is important because it is the first study to correlate EGRF expression with prognosis in rectal cancer patients who received postoperative radiotherapy and fills the gap in the literature in this field.

Conclusion

According to our study, the presence of EGFR expression of 10% or more, LVI, PNI and histologic grade are significantly associated with survival in operated stage 3 rectal cancer patients. These markers can be used as prognostic biomarkers in the follow-up and treatment of these patients.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

Funding: *None*

Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics 2019. CA Cancer J Clin. 2019; 69(1): 7-34.

2. Brown JJ, Asumeng CK, Greenwald D, Weissman M, Zauber A, Striplin J, et al. Decreased colorectal cancer incidence and mortality in a diverse urban population with increased colonoscopy screening. BMC Public Health. 2021; 21(1):1280.

3. Krasinskas AM. EGFR Signaling in Colorectal Carcinoma. Patholog Res Int. 2011; 2011: 932932.

4. Dutta PR, Maity A. Cellular responses to EGFR inhibitors and their relevance to cancer therapy. Cancer Lett. 2007; 254(2): 165-77.

5. Al-Sukhni E, Attwood K, Mattson DM, Gabriel E, Nurkin SJ. Predictors of Pathologic Complete Response Following Neoadjuvant Chemoradiotherapy for Rectal Cancer. Ann Surg Oncol. 2016; 23(4):1177-86.

6. Valentini V, Beets-Tan R, Borras JM, Krivokapić Z, Leer JW, Påhlman L, et al. Evidence and research in rectal cancer. Radiother Oncol. 2008; 87(3): 449-74.

7. Saltz LB, Meropol NJ, Loehrer PJ Sr, Needle MN, Kopit J, Mayer RJ. Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. J Clin Oncol. 2004; 22(7): 1201-8.

8. Douillard JY, Siena S, Cassidy J, Tabernero J, Burkes R, Barugel M, et al. Final results from PRIME: randomized phase III study of panitumumab with FOLFOX4 for first-line treatment of metastatic colorectal cancer. Ann Oncol. 2014; 25(7): 1346-55.

9. Yarom N, Jonker DJ. The role of the epidermal growth factor receptor in the mechanism and treatment of colorectal cancer. Discov Med. 2011; 11(57): 95-105.

10. Huang CW, Chen YT, Tsai HL, Yeh YS, Su WC, Ma CJ, et al. EGFR expression in patients with stage III colorectal cancer after adjuvant chemotherapy and on cancer cell function. Oncotarget. 2017; 8(70): 114663-76.

11. Kim JS, Kim JM, Li S, Yoon WH, Song KS, Kim KH, et al. Epidermal growth factor receptor as a predictor of tumor downstaging in locally advanced rectal cancer patients treated with preoperative chemoradiotherapy. Int J Radiat Oncol Biol Phys. 2006; 66(1): 195-200.

12. Azria D, Bibeau F, Barbier N, Zouhair A, Lemanski C, Rouanet P, et al. Prognostic impact of epidermal growth factor receptor (EGFR) expression on loco-regional recurrence after preoperative radiotherapy in rectal cancer. BMC Cancer. 2005; 5:62.

13. Wu Q, Chen P, Shu C, Chen L, Jin Z, Huang J, et al. Survival outcomes of stage I colorectal cancer: development and validation of the ACEPLY model using two prospective cohorts. BMC Med. 2023; 21(1): 3.

14. Giordano G, Remo A, Porras A, Pancione M. Immune Resistance and EGFR Antagonists in Colorectal Cancer. Cancers (Basel). 2019; 11(8): 1089.

15. Huang CM, Huang CW, Huang MY, Lin CH, Chen CF, Yeh YS, et al. Coexistence of perineural invasion and lymph node metastases is a poor prognostic factor in patients with locally advanced rectal cancer after preoperative chemoradiotherapy followed by radical resection and adjuvant chemotherapy. Med Princ Pract. 2014; 23(5): 465-70.

16. Huh JW, Lee JH, Kim HR, Kim YJ. Prognostic significance of lymphovascular or perineural invasion in patients with locally advanced colorectal cancer. Am J Surg. 2013; 206(5): 758-63.

17. Kılıç Ö, Gültekin Y, Biri İ, Yılmaz AU. The Prognostic Factors on Survival Rates for Patients with Stage II-III Colon Cancer. Turk J Colorectal Dis. 2021; 31: 122-8.

18. Swets M, Kuppen PJK, Blok EJ, Gelderblom H, van de Velde CJH, Nagtegaal ID. Are pathological high-risk features in locally advanced rectal cancer a useful selection tool for adjuvant chemotherapy? Eur J Cancer. 2018; 89: 1-8.

19. Yüksel C, Çulcu S, Gojayev A, Demirci S, Ünal AE. Prognostic Value of Lymphovascular and Perineural Invasion in Colon Cancer. Acta Oncologica Turcica 2021; 54: 166-74.

20. Bayrak M, Altintas Y. Prognostic factors affecting survival in stage 3 colorectal cancers. Annals of Medical Research. 2019; 26(3): 393-7.

How to cite this article:

Murtaza Parvizi, Derya Demir, Engin Kut, Erhan Ergin, Semin Ayhan, Başak Doğanavşargil. *Epidermal growth factor receptor expression and adjuvant chemoradiotherapy in rectal cancer. Ann Clin Anal Med 2023;14(Suppl 1):S86-89*

This study was approved by the Health Sciences Ethics Committee of Ege University (Date: 2017-01-27, No: 16-12.1/1)